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WITNESS my hand this
Twelfth day of September 2003

JR Yabsley

JONNE YABSLEY
TEAM LEADER EXAMINATION
SUPPORT AND SALES

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PROVISIONAL SPECIFICATION

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Invention Title: **Repair of DNA mutagenic damage**

The invention is described in the following statement:

REPAIR OF DNA MUTAGENIC DAMAGE

Field of the Invention

The present invention relates to the use of equol, dehydroequol and isoflav-3-ene and isoflavan compounds in promoting repair of DNA mutagenic damage.

Metallothioneins (MT) are proteins synthesised or over expressed in response to DNA damaging agents e.g. UVR (Hansen et al 1997). In most of the studies in animals and tissue cultures, high does of radiation were used to induce MT, and therefore, it is difficult to extrapolate these results to low level or repeated exposures to UVR in humans (Cai et al 1999). Induced synthesis of MT is considered as one of the mechanisms involved in the adaptive response to low dose UVR exposure, and increased levels of MT appear to be associated with protection from UVR, possibly mediated through scavenging of ROS in the skin (Hanada, et al 1992). As well, MT is implicated in protecting against the immunosuppressive effects of UVR on cell-mediated responses as demonstrated in MT=I and II knockout mice (Reeve, et al 2000). UVR induces immunohistochemically detectable MT in keratinocytes and dermal fibroblasts concurrently with the photoconduction of p53, which suggests the teo protein systems are protective and complimentary in function. MT is detectable in dermal fibroblasts from 2 hours post-UV (Anstey, et al 1996).

Equol, dehydroequol, isofla-3-ene and isoflavan compounds are described in copending provisional patent application 2002950294 which is incorporated herein by reference.

UV exposed skin causes damage in DNA which may give rise to carcinogenesis. The most common tumour in man is the basal cell carcinoma (BCC) followed by squamous cell carcinoma (SCC), and more rarely malignant melanoma.

It has now been found by the applicant that compounds of the present invention, when applied to the skin, result in elevation of metallothioneins production in the skin, particularly the basal layer of irradiated skin.

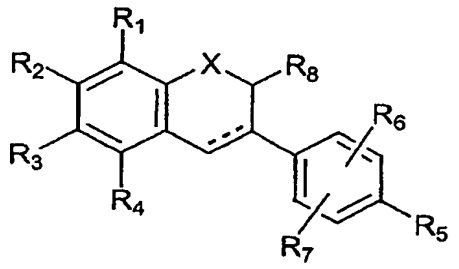
As mentioned above, metallothioneins effect and promote repair of DNA mutagenic damage of skin subject to UV exposure.

In accordance with the present invention there is provided use of equol, dehydroequol, isoflav-3-ene or isoflavan structures for protecting skin from DNA mutagenic damage associated with UV exposure.

In another aspect there is provided use of equol, dehydroequol, isoflav-3-ene or isoflavan structures for the over expression of metallothioneins in the skin, particularly the basal layer of skin.

In accordance with another aspect of this invention there is provided a method for protecting skin from UV induced DNA mutagenic damage which comprises applying to skin a composition containing one or more of equol, dehydroequol, isoflav-3-ene, or isoflavan compounds in admixture with a dermally acceptable carrier.

Isoflav-3-ene and isoflavan compounds may be represented by the general formula (II)

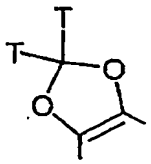


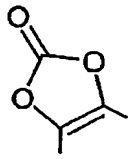
(II)

in which

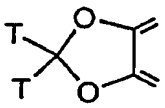
R_1, R_2, R_3 and R_4 are independently hydrogen, hydroxy, OR_9, $OC(O)R_{10}$, $OS(O)R_{10}$, CHO, $C(O)R_{10}$, COOH, CO_2R_{10}, $CONR_{11}R_{12}$, alkyl, haloalkyl, arylalkyl, alkenyl, alkynyl, aryl, heteroaryl, alkylaryl, alkoxyaryl, thio, alkylthio, amino, alkylamino, dialkylamino, nitro or halo, or

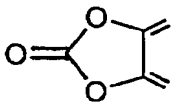
R_3 and R_4 are as previously defined, and R_1 and R_2 taken together with the carbon atoms to which they are attached form a five-membered ring selected from





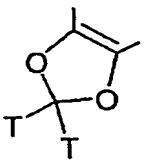
R_1 and R_4 are as previously defined, and R_2 and R_3 taken together with the carbon atoms to which they are attached form a five-membered ring selected from

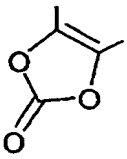




, or

R_1 and R_2 are as previously defined, and R_3 and R_4 taken together with the carbon atoms to which they are attached form a five-membered ring selected from





and

wherein

R_5, R_6 and R_7 are independently hydrogen, hydroxy, OR_9, $OC(O)R_{10}$, $OS(O)R_{10}$, CHO, $C(O)R_{10}$, COOH, CO_2R_{10}, $CONR_{11}R_{12}$, alkyl, haloalkyl, arylalkyl, alkenyl, alkynyl, aryl, heteroaryl, thio, alkylthio, amino, alkylamino, dialkylamino, nitro or halo,

R_8 is hydrogen, hydroxy, alkyl, aryl, amino, thio, $NR_{11}R_{12}$, $CONR_{11}R_{12}$, $C(O)R_{13}$ where R_{13} is hydrogen, alkyl, aryl, arylalkyl or an amino acid, or CO_2R_{14} where R_{14} is hydrogen, alkyl, haloalkyl, aryl or arylalkyl,

R_9 is alkyl, haloalkyl, aryl, arylalkyl, $C(O)R_{13}$ where R_{13} is as previously defined, or $Si(R_{15})_3$ where each R_{15} is independently hydrogen, alkyl or aryl,

R_{10} is hydrogen, alkyl, haloalkyl, amino, aryl, arylalkyl, an amino acid, alkylamino or dialkylamino,

R_{11} is hydrogen, alkyl, arylalkyl, alkenyl, aryl, an amino acid, $C(O)R_{13}$ where R_{13} is as previously defined, or CO_2R_{14} where R_{14} is as previously defined,

R_{12} is hydrogen, alkyl or aryl, or

R_{11} and R_{12} taken together with the nitrogen to which they are attached comprise pyrrolidinyl or piperidinyl,

the drawing "---" represents either a single bond or a double bond, preferably a double bond,

T is independently hydrogen, alkyl or aryl, and

X is O, NR_{12} or S, preferably O,

including pharmaceutically acceptable salts and derivatives thereof.

Dermally acceptable carriers and lotions are well known in the art. The compounds of the present invention may be simply mixed, admixed or blended with suitable carriers to give compositions suitable for application to the skin.

In accordance with another aspects of this invention there is provided a method for the treatment, preventing or amelioration of skin cancer, such as basal cell carcinoma (BCC), squamous cell carcinoma (SCC) and malignant melanoma, which comprises applying to the skin of a subject a composition containing equol, dehydroequol, or an isoflav-3-ene or isoflavan compound of the general formula (II).

In another aspect of this invention there is provided a method for increasing metallothionein production in the skin, such as the basal layer of skin, which comprises applying to skin equol, dehydroequol, isoflav-3-ene or isoflavan compound in association with a dermally acceptable carrier.

The applicant has further found that the compounds according to this invention promote

DNA repair. The promotion of DNA repair may be by one or more of increasing the rate of repair of cyclobutane pyrimidine dimers (CPDs), promoting DNA repair by decreasing P53 expression, and/or by promoting the formation of metallothionein (MT).

The formation of CPD is considered to be an important lethal and mutagenic consequence of UVR exposure (Mitchell et al, 1989; Liardet et al, 2000). Animal models have demonstrated an inverse relationship . between epidermal CPD repair and skin carcinogenesis (Young et al, 1996). The P53 protein (TP53) is expressed after DNA damage by UV irradiation. P53 is a transcription factor which blocks cellular progression from G1 to S phase, thus preventing replication of damaged DNA (Campbell et al, 1993). The P53 protein may act as a tumour promoting agent (Murphey et al, 2001).

This invention will be described with reference to the following, non-limiting example.

Equol was applied to skin before UV irradiation. Twenty-four hours after UV irradiation, MT production was measured. A control lotion was also used containing no equol. This experiment demonstrated that equol caused a statistically significant (P=0.469) elevation in the level of MT in the basal layer of irradiated skin (24 hour post-UV) when compared with unirradiated base line skin (pre-UVR). The vehicle itself did not statistically alter the level of MT in the basal layer of irradiated skin, when compared with unirradiated base line skin.

Figure 1 herewith shows the results of one experiment.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

The reference to any prior art in this specification is not, and should not be taken as an acknowledgment or any form of suggestion that that prior art forms part of the common general knowledge in Australia.

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DATED this 6th day of September, 2002.

Novogen Research Pty Ltd

10 By Its Patent Attorneys

DAVIES COLLISON CAVE

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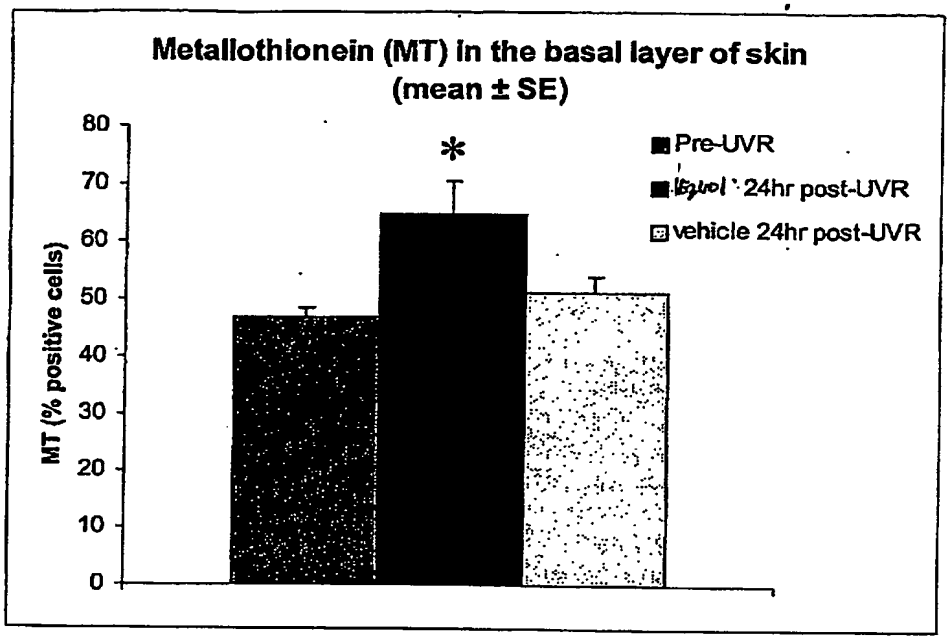
Fig 1



Equol caused a statistically significant (*p = 0.0469) elevation in the level of MT in the basal layer of irradiated skin (24hr post-UV) , when compared with unirradiated baseline skin (Pre-UVR), whereas vehicle did not statistically alter the level of MT in the basal layer of irradiated skin, when compared with unirradiated baseline skin (p = 0.3971)

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